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Original article

## Elevated concentrations of pentraxin 3 are associated with coronary plaque vulnerability

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Received 16 February 2011; received in revised form 18 April 2011; accepted 25 April 2011  
Available online 14 June 2011

### KEYWORDS

Pentraxin 3;  
Coronary plaque;  
Inflammation;  
Atherosclerosis;  
Angiotensin receptor  
blocker

### Summary

**Background:** Inflammation is a critical contributing factor to the development and progression of atherosclerosis. Pentraxin 3 (PTX3) is produced abundantly in atherosclerotic lesions while C-reactive protein (CRP) is mainly produced in the liver. In this study, we investigated whether plasma levels of PTX3 might be a sensitive marker both for the severity of coronary artery disease and vulnerable plaques. Next, we determined whether assays for inflammatory molecules can be used to monitor the therapeutic effects of telmisartan on stabilization of vulnerable atherosclerotic plaques.

**Methods and results:** We measured PTX3 concentrations in the peripheral and coronary sinus plasma of 40 patients with angina pectoris (AP) and 20 control subjects. Next, in 28 patients with AP, we determined the correlation between levels of inflammatory molecules and the computed tomography (CT) density of plaques as a quantitative index of plaque vulnerability. There was no significant difference in peripheral plasma PTX3 concentrations between patients with AP and control subjects, while coronary sinus plasma PTX3 concentrations were significantly higher in AP patients than control subjects. The concentrations of PTX3 in coronary sinus and peripheral plasma correlated with Gensini scores as an index of severity of coronary atherosclerosis. Interestingly, there was a significantly negative correlation between plasma PTX3

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concentrations and CT density ( $r = -0.67$ ,  $p < 0.01$ ). On the other hand, CT density did not correlate with the peripheral plasma concentrations of monocyte chemoattractant protein-1 (MCP-1) or high-sensitivity CRP (hsCRP). Furthermore, telmisartan treatment for 6 months decreased plasma concentrations of PTX3 but not those of MCP-1 or hsCRP in 12 patients with essential hypertension. Multivariate regression analysis revealed that changes in PTX3 levels were independent of blood pressure changes.

**Conclusions:** PTX3 is likely more specific than hsCRP as an indicator of coronary plaque vulnerability that could lead to plaque rupture.

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## Introduction

Inflammation is a critical contributing factor to the development and progression of atherosclerosis, which represents the primary cause of ischemic heart disease and stroke [1,2]. A variety of inflammatory and other biochemical markers potentially related to atherogenesis has been identified [3,4]. However, few inflammatory markers are recognized as established measures of cardiovascular risk prediction. C-reactive protein (CRP) is one of the most actively studied biomarkers. The association between elevated CRP levels and an increased risk for developing cardiovascular events is well established [5]. However, CRP response is triggered by many disorders unrelated to cardiovascular disease.

Pentraxin 3 (PTX3) is the prototypic member of the long pentraxin family and has C-terminal sequence homology with the classic short pentraxins CRP and serum amyloid P component. PTX3 is produced abundantly by various cells in atherosclerotic lesions, including monocytes and macrophages, endothelial cells, vascular smooth muscle cells, fibroblasts, dendritic cells, and adipocytes, while CRP is mainly produced in the liver [6]. These findings suggest that plasma PTX3 concentration reflects local inflammation at the site of the atherosclerotic lesion itself.

In the present study, we compared the roles of plasma PTX3 levels and CRP levels as sensitive markers for the severity of coronary artery disease, evaluated using coronary angiography, and vulnerable plaque, assessed by multidetector-row computed tomography (MDCT). In addition, we determined whether assays of inflammatory molecules can be used to monitor the therapeutic effect of telmisartan—which has been shown to modulate endothelial inflammation and oxidative cell damage *in vitro* [7]—on stabilization of vulnerable atherosclerotic plaques.

## Methods

### Patients

#### Study 1

We studied 40 patients with stable angina pectoris (AP) (mean age: 69 years) and 20 control subjects who had no coronary artery stenosis on angiography (mean age: 68 years). All patients in the control group had no evidence of heart failure or no abnormality in echocardiogram. AP was defined as the occurrence of typical chest pain only during physical exertion and relieved by rest or nitroglycerine and stenosis of more than 50% of the vessel diameter on

elective coronary angiogram. Patients diagnosed as having unstable angina who underwent emergency coronary angiography with recent change in the character, frequency, or severity of anginal pain were excluded. All patients gave written informed consent prior to participation in the study.

The results of coronary angiography were interpreted by 2 senior angiographers who had no knowledge of the results of the biochemical analysis. Based on the primary angiographic results (before coronary intervention), we calculated the Gensini score [8] as the index of the severity of coronary atherosclerosis and subsequently examined the correlation between plasma PTX3 concentrations and Gensini scores.

Blood samples were obtained both from the periphery and from the coronary sinus just before coronary angiography.

#### Study 2

We studied 28 patients (mean age: 67 years) with AP who underwent MDCT (Aquilion, Toshiba Medical, Tokyo, Japan) that showed coronary atherosclerotic plaque.

Blood samples were obtained from the periphery just before MDCT. We investigated whether plasma levels of inflammatory molecules, including PTX3, monocyte chemoattractant protein-1 (MCP-1), and high-sensitivity C-reactive protein (hsCRP), were sensitive markers for the vulnerable plaque characterized by MDCT.

#### Study 3

Next, we determined whether assays for inflammatory molecules including PTX3 can be used to monitor the therapeutic effect of telmisartan—which has been shown to modulate endothelial inflammation *in vitro*—on stabilization of vulnerable atherosclerotic plaques in 12 patients with essential hypertension (mean age: 69 years). As the control group, 8 patients receiving the calcium channel blocker, amlodipine, were also studied.

## Biochemical analysis

Plasma concentrations of PTX3 were measured by enzyme-linked immunosorbent assay, using the F(ab')<sub>2</sub> fragment of a monoclonal anti-human PTX3 antibody, PPMX0104 (Perseus Proteomics Inc., Tokyo, Japan) as the capture antibody and PPMX0105 (Perseus Proteomics Inc.) conjugated with horseradish peroxidase as the detection antibody, based on a previously described method [9]. Plasma MCP-1 concentrations were determined by enzyme-linked immunosorbent

**Table 1** Patient characteristics.

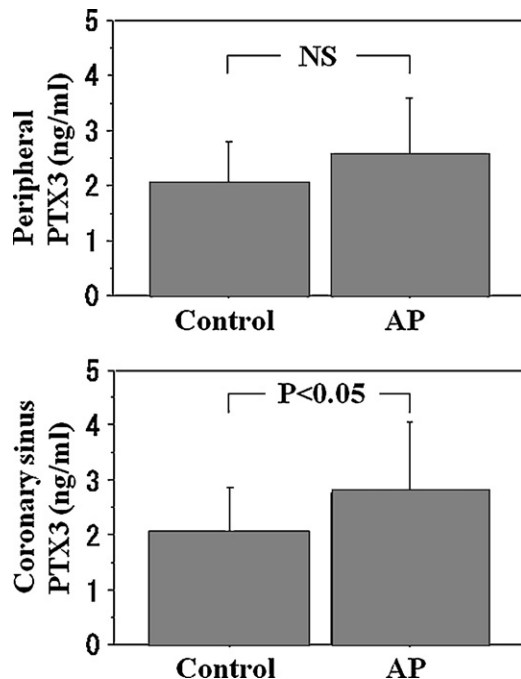
	Angina pectoris	Control
<i>n</i>	40	20
Age (years)	69 ± 9	68 ± 9
Male/female	27/13	12/8
Smoking ( <i>n</i> )	17 (43%)	5 (25%)
Hypertension ( <i>n</i> )	24 (60%)	8 (40%)
Diabetes mellitus ( <i>n</i> )	14 (35%)	3 (15%)
LDL-C (mg/dl)	127 ± 33	121 ± 33
HDL-C (mg/dl)	47 ± 12	54 ± 13
Triglycerides (mg/dl)	138 ± 52	120 ± 50

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

assay (R&D Systems, Abingdon, UK). Serum hsCRP concentrations were measured by the latex agglutination method.

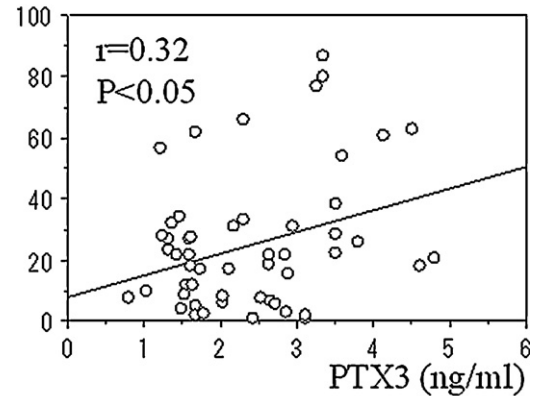
### MDCT images (Study 2)

MDCT was performed immediately after the blood sampling using an Aquilion Advanced Multidetector-row CT, based on a previously described method [10]. An experienced cardiologist and an experienced radiologist evaluated coronary CT scans. Curved and cross-sectional images of coronary artery wall plaques were obtained, and CT values were measured using circular regions of interests (ROIs) on the cross-sectional multiplanar reconstruction (MPR) images. In

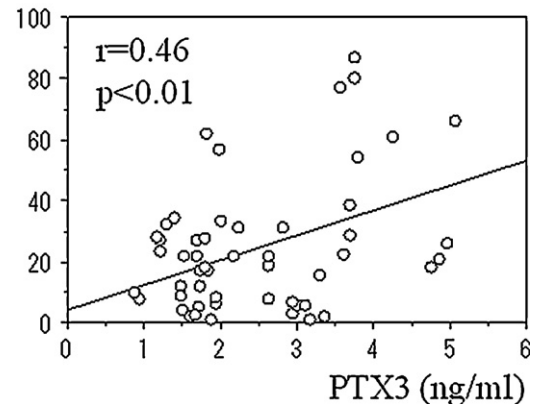


**Figure 1** Plasma pentraxin 3 (PTX3) concentrations in the periphery and coronary sinus of control subjects and patients with angina pectoris (AP). There was no significant difference in peripheral plasma PTX3 concentrations between AP patients and control (top). Plasma PTX3 concentrations in the coronary sinus were higher in patients with AP than in control subjects (bottom).

### Gensini Score



### Gensini Score



**Figure 2** Plasma pentraxin 3 (PTX3) concentrations and coronary atherosclerosis. Peripheral plasma PTX3 concentrations were significantly correlated with Gensini scores (top). A stronger correlation was seen between Gensini scores and the concentrations of PTX3 in coronary sinus plasma (bottom).

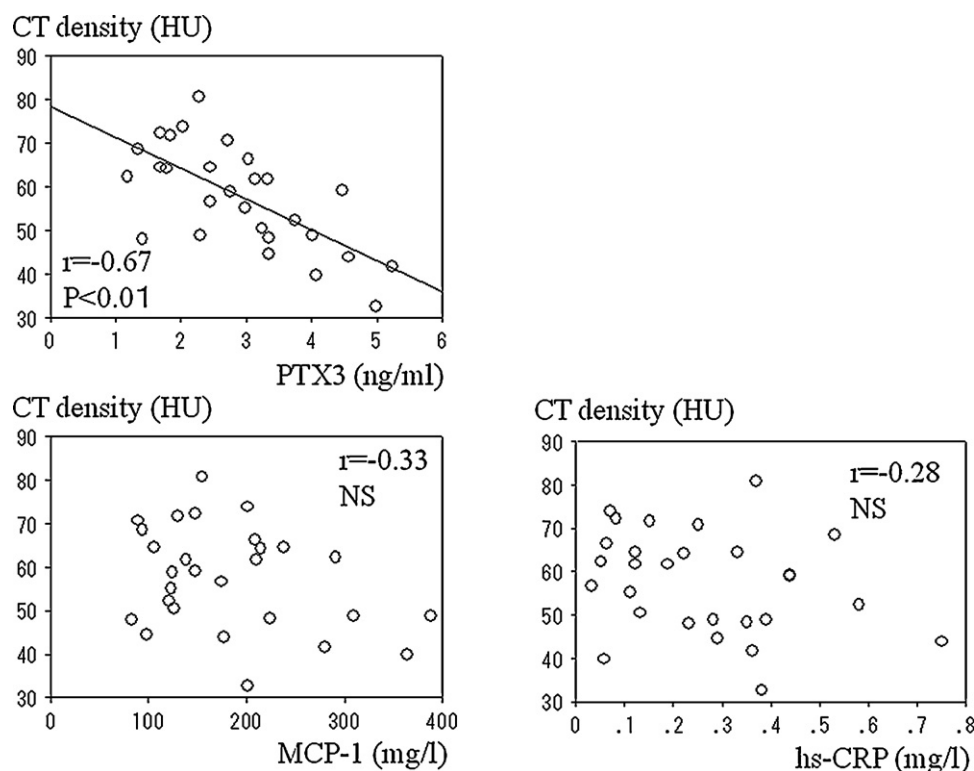
AP patients who had multiple plaques in the culprit segment, the minimum CT value of the plaque at the stenotic lesion was used for analysis.

### Administration of telmisartan (Study 3)

Telmisartan was prescribed once per day at a dose of 40 mg immediately after breakfast for 6 months. The target systolic/diastolic blood pressure was <130/85 mmHg in patients under 74 years old. In those over age 75 years, the target blood pressure was 140/90 mmHg. In patients in whom the systolic/diastolic blood pressure did not reach the target blood pressure, the telmisartan dose was increased to 80 mg. Before and after telmisartan administration, biochemical analysis (PTX3, MCP-1, hsCRP) was performed.

### Statistical analysis

Values are expressed as the means ± S.D. Comparisons between two groups were performed using a Student's unpaired *t*-test for continuous variables and a chi-square test for categorical variables. Correlations between two parameters were assessed using simple linear regression.



**Figure 3** Correlation between computed tomography (CT) density and peripheral plasma pentraxin 3 (PTX3) concentrations. Peripheral plasma PTX3 concentrations negatively correlated with CT density (top). In contrast, no correlation was seen between CT density and peripheral plasma concentrations of monocyte chemoattractant protein-1 (MCP-1) or high-sensitivity C-reactive protein (hsCRP) (bottom).

## Results

### Patient characteristics

Table 1 summarizes the characteristics of each study group. There were no significant differences in age, gender, incidence of smoking, hypertension, diabetes, or levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, or triglycerides between AP patients and controls. The lesion site of coronary artery disease was the left anterior descending artery in 23 patients (57.5%), left circumflex artery in 12 cases (30.0%), and right coronary artery in 19 (47.5%).

### Plasma PTX3 concentrations and coronary atherosclerosis

There was no significant difference in peripheral plasma PTX3 concentrations between AP patients and controls ( $2.6 \pm 1.0$  ng/ml vs.  $2.1 \pm 0.7$  ng/ml) (Fig. 1, top). Coronary sinus plasma PTX3 concentrations were higher in AP patients than in control subjects ( $2.8 \pm 1.3$  ng/ml vs.  $2.1 \pm 0.8$  ng/ml) (Fig. 1, bottom).

PTX3 concentrations in peripheral plasma significantly correlated with Gensini scores ( $r = 0.32$ ,  $p < 0.05$ ) (Fig. 2, top). This correlation was even stronger for PTX3 in coronary sinus plasma ( $r = 0.46$ ,  $p < 0.01$ ) (Fig. 2, bottom). Furthermore, there was more strong correlation between the delta

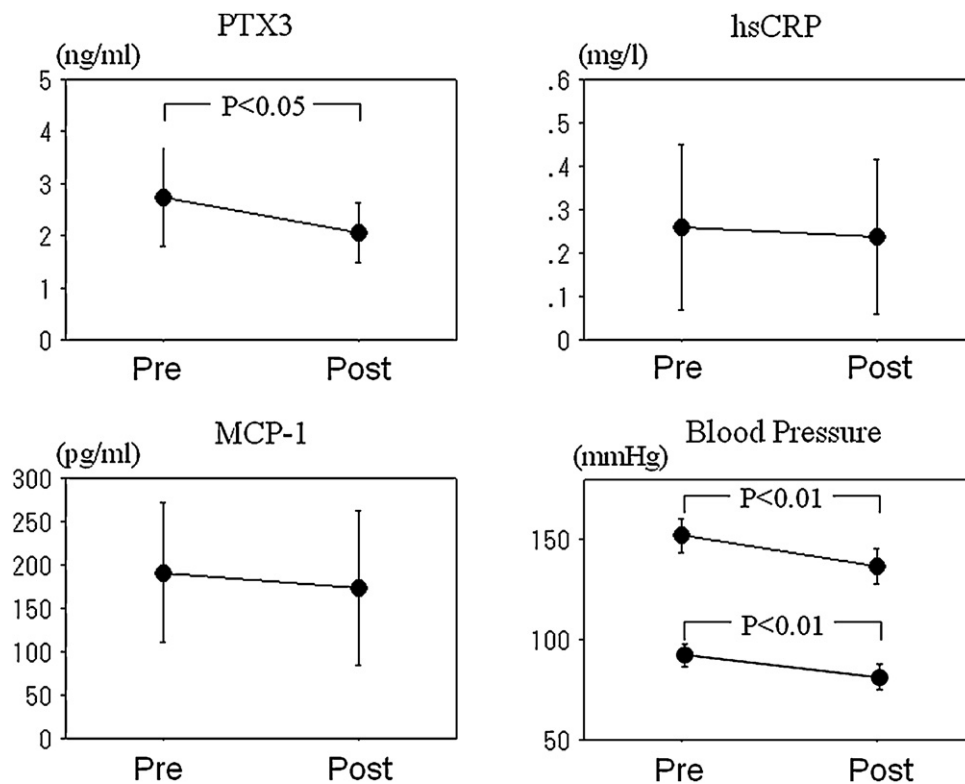
PTX3 (coronary sinus PTX3 – peripheral PTX3) and Gensini score ( $r = 0.49$ ,  $p < 0.01$ ).

### Correlation between CT density and peripheral plasma PTX3 concentrations

There was a significantly negative correlation between plasma PTX3 concentrations and CT density ( $r = -0.67$ ,  $p < 0.01$ ) (Fig. 3, top). On the other hand, peripheral plasma concentrations of MCP-1 and hsCRP did not correlate with CT density (NS) (Fig. 3, bottom).

### Changes in plasma levels of inflammatory markers before and after 6 months of telmisartan treatment

The pretreatment systolic and diastolic blood pressures were  $152 \pm 9$  and  $90 \pm 6$  mmHg, respectively. After telmisartan administration, these decreased significantly to  $137 \pm 9$  and  $79 \pm 6$  mmHg, respectively ( $p < 0.01$ ) (Fig. 4). Plasma PTX3 concentrations decreased significantly from  $2.75 \pm 0.95$  ng/ml to  $2.06 \pm 0.58$  ng/ml after telmisartan treatment ( $p < 0.05$ ) (Fig. 4). On the other hand, plasma concentrations of MCP-1 and hsCRP did not change following telmisartan administration (Fig. 4). Multivariate regression analysis revealed that changes in PTX3 levels were independent of variations in blood pressure, lipid profiles, and hemoglobin A1c levels. While comparable blood pressure lowering was achieved in patients treated with amlodip-



**Figure 4** Changes in plasma levels of inflammatory markers before and after 6 months of telmisartan treatment. After telmisartan administration, both systolic and diastolic blood pressures were significantly decreased ( $p < 0.01$ ). Plasma concentrations of pentraxin 3 (PTX3) decreased significantly from  $2.75 \pm 0.95$  ng/ml to  $2.06 \pm 0.58$  ng/ml after telmisartan treatment ( $p < 0.05$ ). On the other hand, plasma concentrations of monocyte chemoattractant protein-1 (MCP-1) and high-sensitivity C-reactive protein (hsCRP) did not change with telmisartan administration.

ine (from  $154 \pm 9/91 \pm 6$  mmHg to  $140 \pm 7/81 \pm 6$  mmHg), plasma concentrations of PTX3, MCP-1 and hsCRP did not change after the administration of amlodipine.

## Discussion

In the present study, we demonstrate the direct relationship between plasma PTX3 concentrations and coronary plaques. Furthermore, we report that the plasma PTX3 level may be a sensitive marker for vulnerable plaques.

Our findings agree with previous basic and clinical research. We found that PTX3 concentrations in coronary sinus plasma or delta PTX3 correlated with Gensini scores more strongly than those in peripheral plasma, suggesting that PTX3 reflects the degree of local inflammation in coronary atherosclerosis; it also agrees with previous basic research in which immunohistochemistry of advanced atherosclerotic lesions showed an elevated PTX3 expression in neutrophils and macrophages [11,12] and with clinical studies demonstrating that patients with acute myocardial infarction and unstable angina had elevated plasma PTX3 concentrations [9,13].

Interestingly, peripheral plasma PTX3 concentrations strongly correlated with CT density, while MCP-1 and hsCRP concentrations did not. A previous report showed that MDCT can detect coronary artery plaques by demonstrating good agreement between the CT density of plaques and their

echogenicity as determined by intracoronary ultrasound; low-density plaques on CT correspond to lipid core-containing coronary plaques, while medium-density plaques correspond to fibrous coronary plaques [14]. Another previous study showed that patients with acute coronary syndrome had low-density plaques on CT as compared with those shown in patients with stable angina, and that plaque density was lower in culprit segments than in non-culprit segments in patients with acute coronary syndrome [15]. Thus, MDCT may potentially predict future cardiac events. These findings, together with our results, suggest that plasma PTX3 concentrations reflect coronary plaque vulnerability that leads to plaque rupture more specifically than CRP or MCP-1 concentrations.

In the present study, the concentrations of neither hsCRP nor MCP-1 were correlated with plaque density on CT, suggesting that CRP and MCP-1 may be relatively poor predictors of vulnerable plaques in comparison with PTX3. This finding does not agree with previous large-scale studies that showed CRP as a strong and independent predictor of atherosclerotic risk in individuals with and without established cardiovascular disease [16]. CRP likely reflected other systemic inflammation such as transient infection, and the resulting overestimation could have a significant effect on the specificity of CRP for evaluating vulnerable coronary plaques in a limited number of patients. Plasma MCP-1 concentrations were reported to be higher in patients with unstable angina than those with stable angina [17]. While



MCP-1 mediates monocyte recruitment and entry into vessel walls at sites of atherosclerosis, PTX3 is associated with macrophage differentiation into foam cells and with neutrophil infiltration into atherosclerotic plaques [12]; these differences might lead to the result of our study showing that PTX3 was more specifically related to plaque instability than MCP-1. Neutrophils as well as foamy macrophages have been shown to be major sources of PTX3, contributing to the process of coronary atherosclerotic plaque activation [12] and potentially leading to plaque rupture, thrombosis, and ischemia.

It has been demonstrated that the inhibition of the renin–angiotensin system by an angiotensin-converting enzyme inhibitor or an angiotensin II receptor type 1 antagonist reduces cardiovascular disease [18,19]. In particular, telmisartan has been shown to have peroxidase proliferator-activated receptor gamma activating effects independent from its angiotensin II-receptor blocking abilities [20] and is suggested to improve insulin sensitivity, leading to a reduction of the risk for atherosclerosis. In addition, our recent study showed that telmisartan decreased pulse-wave velocity [21], suggesting that this agent improves vascular endothelial function. However, there has not been a concise and specific biomarker that reflects the degree of atherosclerosis and the effect of pharmacologic intervention in the same way, for instance, that brain natriuretic peptide reflects the degree of heart failure. Therefore, we investigated whether telmisartan decreased the level of PTX3 and whether PTX3 level is a surrogate marker for atherosclerosis. In the present study, we showed that plasma PTX3 concentrations decreased significantly after treatment with telmisartan and changes in PTX3 levels were independent of blood pressure changes, suggesting that PTX3 is likely a useful marker for monitoring the anti-plaque effect of telmisartan.

Our study has several limitations. First, our sample size was small. We measured the plasma concentrations of PTX3, MCP-1, and hsCRP in only a limited number of patients, and were therefore unable to definitively exclude the predictive value of MCP-1 and hsCRP regarding vulnerable plaques. Second, we used only MDCT for the evaluation of coronary vulnerable plaques. To confirm the relationship between PTX3 and vulnerable plaques, in further studies we should use other examination methods for coronary vulnerable plaques, such as intracoronary ultrasound. Third, we did not perform sampling from coronary sinus in Study 2 because the different timings of MDCT and sampling from coronary sinus (at coronary angiography) may have some effects on the results.

In conclusion, we found that there was a significantly negative correlation between plasma PTX3 concentrations and CT density, but no correlation between CT density and plasma concentrations of MCP-1 and hsCRP. Thus, PTX3 likely reflects coronary plaque vulnerability that leads to plaque rupture more specifically than MCP-1 or CRP.

## Acknowledgments

The authors thank all of the following for their assistance and contributions to the study: Etsuko Uematsu, Kazue Ishikawa, and Yoshie Miki.

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